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Comparison of two independent scoring techniques for spot variation in *Maniola jurtina* (L.) and the consequences of some differences

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Eight samples (total $N = 298$) of female meadow brown butterflies *Maniola jurtina* were scored independently by P. M. Brakefield and W. H. Dowdeswell for hindwing spot-number and spot-combination. Spot-size was measured by P.M.B. W.H.D scored 37% few spots overall than P.M.B. ($N = 554$). This resulted in some marked differences in spot-frequency distributions. There was a rather narrow threshold of spot-size above which nearly all spots were scored by both of us and below which most spots were only scored by P.M.B. When the data of P.M.B. are transformed by excluding all spots below a threshold a close correspondence of spot-frequencies is found. Relative differences between samples tend to be maintained in the untransformed data. The consequences of the differences in scoring techniques are discussed.

KEY WORDS:—*Maniola jurtina* – scoring technique – comparison – spot-frequency distribution – consistency – spot-size – threshold – transformed – quantitative character.

CONTENTS

INTRODUCTION

The undersides of the hindwings of the meadow brown butterfly, *Maniola jurtina*, exhibit variation in the number of small submarginal spots (Dowdeswell & Ford, 1952). This variation has been extensively studied for over 30 years (see reviews by Ford, 1975; Dowdeswell, 1981; Brakefield, 1984). Differences

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between populations throughout the species's range have been identified with reference to the spot-frequency distribution within them and to the mean number of spots or spot-average. The spots are found at each of five (rarely six) defined positions on the hindwing at which they may be present or absent. Only a limited number of the possible spot-combinations occur. McWhirter & Creed (1971) devised a costality index describing the proportion of spots which are positioned costally as a measure of the spot-placing variation within populations. At each position on the hindwing the spots vary in size in a quantitative manner (Brakefield, 1984). The work on *M. jurtina* has been concerned with a number of problems in ecological genetics, including the evolution of adaptations to differing environments (e.g. Ford, 1975).

Until the early 1970s, the investigation was carried out almost exclusively by a group of workers based at the University of Oxford and led by E. B. Ford and W. H. Dowdeswell. Other workers have become actively, but largely independently, involved during the last decade (see e.g. Brockie, 1972; Frazer & Willcox, 1975; Tudor & Parkin, 1979; Brakefield, 1984). Comparisons of some of the samples obtained by these workers with those described earlier by the group based at Oxford (e.g. Dowdeswell & McWhirter, 1967) suggest that changes in spot variation have occurred within some geographical zones or populations (see Brakefield, 1984). Observations of this kind underline the importance of ensuring the consistency of the scoring techniques employed. We therefore report here the results of a test which show that whilst there can be a close correspondence between different scorers there may also be important differences which must be taken into account when comparisons are made. Failure to do this could lead to misleading conclusions.

MATERIALS AND METHODS

Samples of female *M. jurtina* from eight populations in England were scored for spot-variation on the left hindwings by P. M. Brakefield (P.M.B.). The sample size ranged from 29 to 51 with a mean of 37.25 and a total of 298. The width of each spot along the wing internervules was measured using a binocular microscope fitted with a micrometer (1 unit $\approx$ 0.054 mm). Each butterfly was stored in a numbered envelope. They were subsequently scored independently by W. H. Dowdeswell (W.H.D.). He recorded the number and position of the spots according to the technique used by each member of E. B. Ford's group.

RESULTS

Initial inspection of the two data sets indicated that considerably fewer spots were recorded as present by W.H.D. than by P.M.B. Thus, of the total of 554 spots scored by P.M.B. only 347 (63%) were also scored by W.H.D. (no spots were recorded only by W.H.D.). Table 1 shows that this difference has a marked effect on the spot-frequency distributions for the combined sample. Whilst the distribution for W.H.D.'s data is unimodal at 0 spots, that for P.M.B.'s shows similar frequencies at 0, 1 and 3 spots with a mode at 2 spots. There is a corresponding difference in spot-average and some influence on the costality index for spot-placing (Table 1). Of the 298 females, only 144 (48.3%)

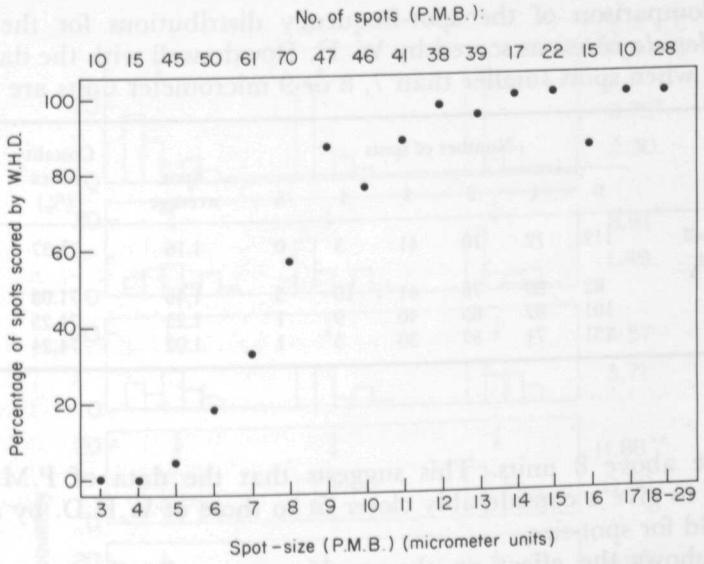

Figure 1. The relationship between the size of spots in the combined sample of *Maniola jurtina* measured by P. M. Brakefield and the proportion of the spots scored by W. H. Dowdeswell.

were scored as the same spot-number (and spot-placing combination). An extra spot was scored by P.M.B. in 105 (35.2%) specimens and an additional 2 or 3 spots in the other 49.

The nature of the difference in scoring technique can be examined through analysis of the measurements made by P.M.B. of spot-size. Figure 1 shows the relationship between these data and those of W.H.D. for spot-presence. The smallest spots of 3 to 4 or 5 micrometer units are not scored by W.H.D. These represent only a small number (in the case of 3 units sometimes only one) of black wing scales at the defined positions for spots on the hindwings. The fact that such spots are not recorded by W.H.D. corresponds with the original description of scoring by Dowdeswell & Ford (1952) which states that, "a spot was regarded as absent if it could not be distinguished from a black scale which might have occurred anywhere on the wing". However, a proportion of larger spots as measured by P.M.B. were also not scored by W.H.D. (Fig. 1). These account for much of the difference in the spot-frequency distributions (Table 1). The range in spot-size for which the probability of a difference in scoring is substantial is rather narrow (Fig. 1). Thus only 3% of spots recorded by W.H.D. were below 7 units in size and only 13% of those recorded only by

Table 1. Comparison of spot-frequency distributions for the combined sample of *Maniola jurtina* scored independently by each recorder

Recorder	Number of spots						Spot average	Costality index (%)	Chi-square (4 d.f.)
	0	1	2	3	4	5			
W. H. Dowdeswell	112	72	70	41	3	0	1.16	75.37	49.49***
P. M. Brakefield	60	57	87	59	29	6	1.86	68.70	

*** $P < 0.001$.

excluding spots below 8 size units there is a close correspondence in the spot-frequency distributions with no differences between those for the individual samples (Fig. 2B & C). Furthermore, there is a significant Spearman rank correlation between the spot-averages for the two series of spot-frequency distributions ($r_s = 0.929$, $P < 0.05$ and see Fig. 2). The heterogeneity between the first four samples and the last four for W.H.D.'s data is also evident in these transformed data ($\chi^2 = 15.99$, 3 d.f., $P < 0.01$).

DISCUSSION

The results of this analysis show that to compare our data for spot-number and spot-placing it is necessary to transform those of P.M.B. using measurements of spot-size. However, the untransformed data of P.M.B. reflect the relative differences between populations evident from analysis of W.H.D.'s data (see Fig. 2). Thus, the groupings of the first four and last four samples are again heterogeneous ($\chi^2 = 10.40$, 4 d.f., $P < 0.05$) as are the eight individual samples ($\chi^2 = 51.62$, 28 d.f., $P < 0.01$).

The basic difference between the scoring techniques employed by us is that whilst the complete continuous distribution of spot-size is reflected by the technique of P.M.B., a form of threshold is used by W.H.D. such that the lower tail of the distribution represents a wider range of phenotypes.

The possibility of some variability between scorers was taken into account when the scoring technique employed by E. B. Ford and W. H. Dowdeswell was developed (see Dowdeswell & Ford, 1952). Thus, they use a form of consensus scoring where if there is doubt about the spotting of any specimen at least two workers are available to give an opinion. The effect of this is to reduce variations in interpretation of the type analysed in the present study.

Our results illustrate that for quantitative characters of the type studied in *M. jurtina* it is necessary to standardize the scoring techniques employed by different workers particularly when the absolute size of the character is not measured and where there may be some question as to what constitutes expression of the character. Any differences between the techniques must be taken into account when the data are interpreted. If the present data sets had been obtained independently and without co-operation it would have been concluded that they represented samples from different groups of populations although ones exhibiting similar patterns of relative differences. These observations are also relevant to the numerous studies of variation in spot patterns in other species of Lepidoptera (Brakefield, 1984).

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